

ORIGINAL ARTICLE

Hepatoprotective activity of Methanol extract of *Francoeuria undulata* against Paracetamol induced Hepatotoxicity in rats

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ABSTRACT

Many hepatoprotective herbal preparations have been recommended in alternative systems of medicine for the treatment of hepatic disorders. No systematic study has been done on protective efficacy of *Francoeuria undulata* to treat hepatic diseases. *Francoeuria* is a diverse genus of the Asteraceae family. Therefore, protective action of *Francoeuria undulata* aerial parts extract was evaluated by us in an animal model of hepato-toxicity induced by paracetamol. *Francoeuria undulata* aerial parts powder (25 g) was extracted with 250 ml 80% methanol. The 80, 150 mg/kg concentrations of extract were prepared. Wistar albino rats were divided into 6 groups: 1) control, 2) paracetamol (100 mg/kg), 3 and 4) paracetamol (100 mg/kg) + extract (80, 150 mg/kg), 5 and 6) extract (80, 150 mg/kg). The blood samples were collected and liver markers were assayed in serum. The results were analyzed using ANOVA statistical to analyses of the treatments. Levels of marker enzymes such as alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT) and lactate dehydrogenase (LDH) were increased significantly in paracetamol treated rats (group 2). *Francoeuria undulata* aerial parts extract brought about a significant decrease in the activities of all these factors. *Francoeuria undulata* aerial parts extract treatment at concentration 150 mg/kg led to the recovery of these levels to near normal.

The present observations suggested that the treatment with *Francoeuria undulata* aerial parts extract enhance the recovery from paracetamol induced hepatic damage due to its anti-oxidant and hepatoprotective property.

Keywords: *Francoeuria undulata*, Liver, Paracetamol.

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INTRODUCTION

The use of herbal medicines in Asia demonstrates a long history of human interactions with the environment [1]. Systematic experiments of folk medicine plants may result in the discovery of new effective compounds. Many attempts have been made to discover new effective compounds from various kinds of sources such as micro-organisms, animals, and plants [2]. Although the biosynthesis of effective compounds in plants has strong genetic control, environmental conditions also use a significant impact on their production rates [3, 4]. Many studies have been published concerning several properties such as antibacterial, antitumor, antifungal, and antioxidant activity in some species in plants [5, 6].

Liver is an important organ which it has a wide range of functions, including detoxification, protein synthesis and production of biochemicals necessary for digestion, glycogen storage, decomposition of red blood cells, hormone production [7]. The liver is exposed to many oxidative agents, thus antioxidant compounds are useful for liver health [8].

Free radicals are highly reactive molecules that are produced by biochemical redox reactions occurring in natural process of cell metabolism and oxidative stress occurs when the free radicals are produced in large amounts or the antioxidant levels are low, as a result, the free radicals may cause lipid peroxidation and damage to cellular structures, nucleic acids, proteins and lipids. [9, 10].

A many of natural antioxidants have already been isolated from various kinds of plant materials such as vegetables, fruits, leaves, oilseeds, cereal crops, roots, spices, and herbs [11]. Dietary antioxidants can stimulate cellular defenses and help to inhibit cellular components against oxidative damage [12]. Plants contain a various group of phenolic compounds including simple phenolics, phenolic acids, anthocyanins, hydroxycinnamic acid derivatives, and flavonoids. All the phenolic classes have the structural requirements of free radical scavengers and have potential as food antioxidants [13]. Factors inducing the antioxidant activity of plant phenolics include position and degree of hydroxylation, stability of the phenol to food processing operations, polarity, solubility, reducing potential, and stability of the phenolic radical [14].

Francoeuria undulata (L.) LACK. is a perennial aromatic herb, from the Compositae family (Tribe: Inuleae), producing small bright yellow flowers. This plant is often synonymous with two species of the genus *Pulicaria*, i.e. *P. undulata* (L.) C.A. Mey. and *P. crispa* (Forssk.) olive, which are often contained under the genus *Francoeuria* (15, 16). This species is found distributed in Saudi Arabia, Kuwait, Iran, Iraq, Egypt, Afghanistan, Pakistan, India and parts of northern and western tropical Africa [17]. Anticancer and antiviral properties of this plant have been reported in different studies [18, 19]. Barry *et al.* (1970) and Gerging *et al.* (1991) studied antibacterial activity of ethanol extract of this plant on several standard bacterial strains. They showed strong antibacterial activity of this extract on *Escherichia coli* (ATCC 25923), *Staphylococcus aureus* (ATCC 25923), *Proteus vulgaris* (ATCC 6380) and *Klebsiella pneumoniae* (ATCC 1312) [20, 22]. Bohlmann *et al.*, (1979), demonstrated that *P. crispa* aerial parts have a new pseudoguaianolide epoxide and a sesquiterpene lactone compound [23]. Abdel-Mogib *et al.*, (1990) and Starvi *et al.*, (2008) studies have indicated that the extractions of the *Pulicaria* species are rich sources of the sesquiterpene lactones [24, 25].

In the present work,, methanolic extracts of aerial parts of *Francoeuria undulata* plant and paracetamol poison were screened on several liver factors.

MATERIALS AND METHODS

Plant materials

The aerial parts of *Francoeuria undulata* was collected from northeast of Esfahan province (Ardestan), Iran, in September 2015. The voucher specimen was deposited at the herbarium of the Research-Institute of Esfahan Forests and Rangelands. The aerial parts were air-dried under shade and ground into fine powder using electric blender, then, 25 gr of aerial parts powder were extracted with 250 ml methanol 80% by Maceration method. In this way, a mixture of methanol and aerial parts powder were placed on the shaker for 72 hours, then this was smoothed by the filter paper. The residue was evaporated at room temperature and the dried extract was stored at 4°C until used. The extract was dissolved in phosphate buffered saline at concentrations of 80, 150 mg/kg body weight.

Paracetamol was procured from Raha Company. Then, it was dissolved phosphate buffered saline at concentration of 100 mg/kg body weight.

Animals

Adult male wistar rats (200-250 g) were obtained from Iran Pastor Institute and divided into 6 groups of eight animals each (48 rats). They were maintained under controlled temperature, 12 h light/12 h dark conditions for 1 week before the start of the experiments for adaptation to laboratory conditions. The procedures in this study were carried out in accordance with the institution's scientific procedures for animals and was approved by the Institutional Animal Care. The animals were randomly divided into the groups that were gavaged. These groups include the 1.control group that received phosphate buffered saline, 2.paracetamol treated group (100 mg/kg), 3. paracetamol (100 mg/kg)+extract (80 mg/kg), 4. paracetamol (100 mg/kg)+extract (150 mg/kg), 5 and 6.extract treated groups (80 and 150 mg/kg) respectively. The animals were treated with paracetamol and extract for 20 days (once daily after two day).

The animals were anesthetized by injection with Ketamine (0.07 ml/100 g body weight) and the blood samples were collected 1 days after the last gavage. The biochemical parameters such as AST, ALT, ALP, LDH, and GGT were assayed using autoanalyzer (902 Hitachi Automatic Analyzer, Roche, India) [26].

Statistical analysis

The presented data included means of three separate experiments \pm SD. In order to analyze the data, SPSS software and ANOVA test were used. Thus, the statistical significance between means was evaluated with a LSD test. P values less than 0.05 were considered to be statistically significant.

RESULTS

According to the results, significant changes were observed in the mean value AST, ALP, GGT, LDH. The rate of these parameters changed between groups and these changes were significant. Whereas, the rate of AST, ALP, GGT, LDH decreased significantly in the groups 3, 4, 5, 6 (paracetamol+extract 80 mg/kg, paracetamol+extract 150 mg/kg, extract 80mg/kg, extract 150 mg/kg) in compared with group 2 (paracetamol) (P<0.05).

Analysis of data on LDH showed that LDH decreased significantly in the groups 4, 6 (paracetamol+extract 150 mg/kg, extract 150 mg/kg) in compared with group 2 (paracetamol) (P<0.05) (Figure 1), whereas, significant changes were observed in the groups 4 (paracetamol+extract 150 mg/kg) in compared with group 6 (extract 150 mg/kg) (P<0.05).

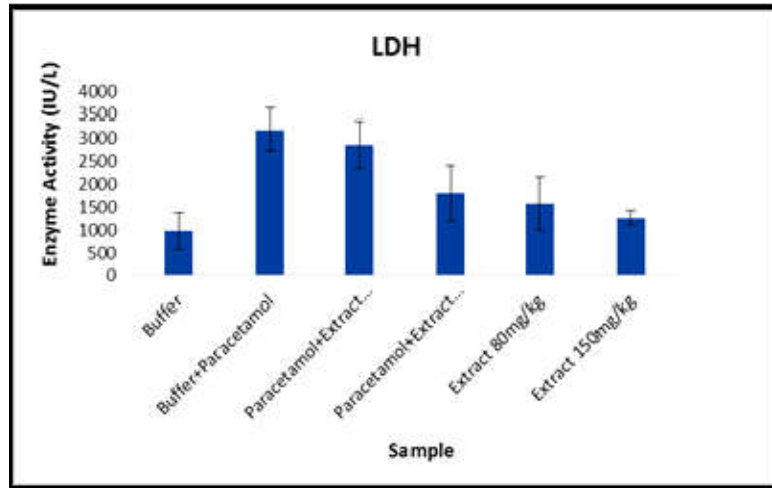


Figure 1. Significant reduction of LDH activity (IU/L) of paracetamol+extract 150 mg/kg and extract 150 mg/kg in compared with paracetamol. Bars are least significant differences where p < 0.05.

Analysis of data on GGT showed that GGT decreased significantly in the groups 4, 6 (paracetamol+extract 150 mg/kg, extract 150 mg/kg) in compared with group 2 (paracetamol) (P<0.05) (Figure 2), whereas, no significant changes were observed in the groups 4 (paracetamol+extract 150 mg/kg) in compared with group 6 (extract 150 mg/kg) (P>0.05).

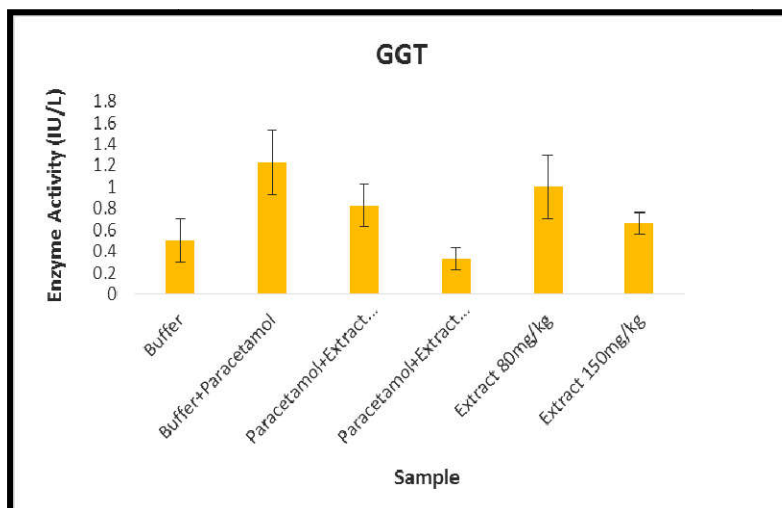


Figure 2. Significant reduction of GGT activity (IU/L) of paracetamol+extract 150 mg/kg and extract 150 mg/kg in compared with paracetamol. Bars are least significant differences where p < 0.05.

Analysis of data on ALT showed that, no significant changes were observed in the mean value of ALT,. Whereas, the rate of ALT decreased in the groups 5, 6 (extract with concentration of 80, 150 mg/kg) in compared with group 2 (paracetamol) ($P > 0.05$) (Figure 3).

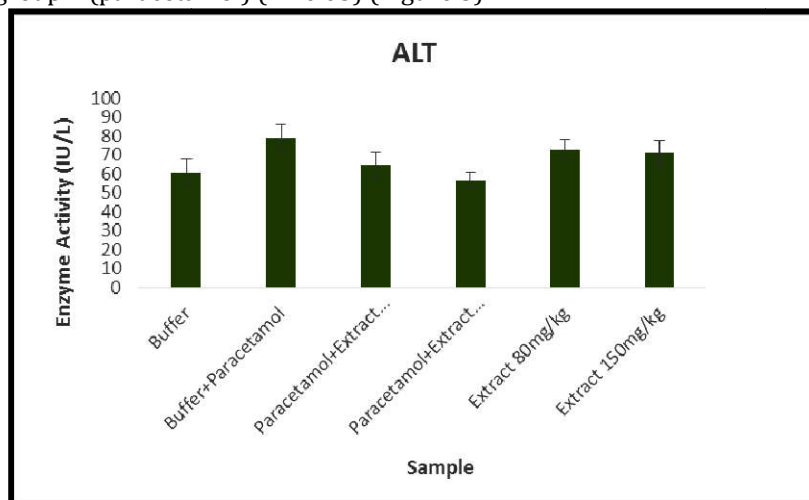


Figure 3. Not significant reduction of GGT activity (IU/L) of extract 80 mg/kg and extract 150 mg/kg in compared with paracetamol. Bars are least significant differences where $p > 0.05$.

Analysis of data on AST showed that AST decreased significantly in the groups 4, 6 (paracetamol+extract 150 mg/kg, extract 150 mg/kg) in compared with group 2 (paracetamol) ($P < 0.05$) (Figure 4), whereas, no significant changes were observed in the groups 4 (paracetamol+extract 150 mg/kg) in compared with group 6 (extract 150 mg/kg) ($P > 0.05$).

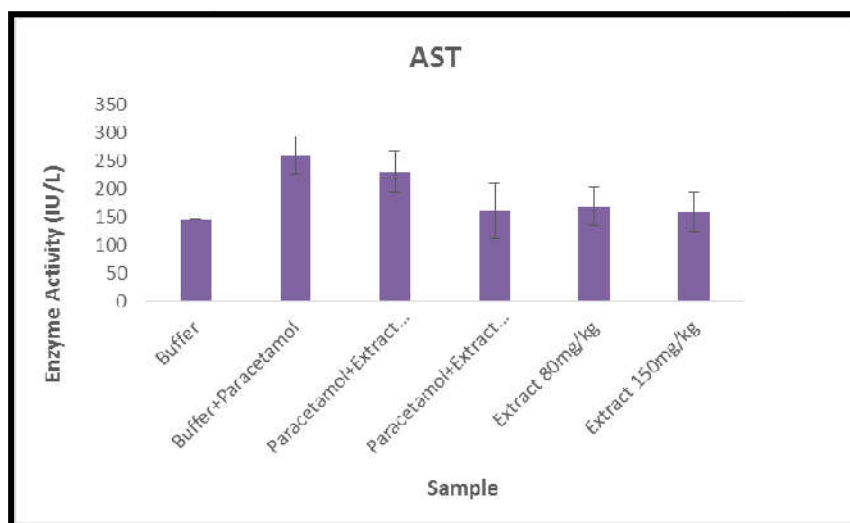


Figure 4. Significant reduction of GGT activity (IU/L) of paracetamol+extract 150 mg/kg and extract 150 mg/kg in compared with paracetamol. Bars are least significant differences where $p < 0.05$.

Analysis of data on ALP showed that ALP decreased significantly in the groups 4, 6 (paracetamol+extract 150 mg/kg, extract 150 mg/kg) in compared with group 2 (paracetamol) ($P < 0.05$) (Figure 5), whereas, significant changes were observed in the groups 4 (paracetamol+extract 150 mg/kg) in compared with group 6 (extract 150 mg/kg) ($P < 0.05$).

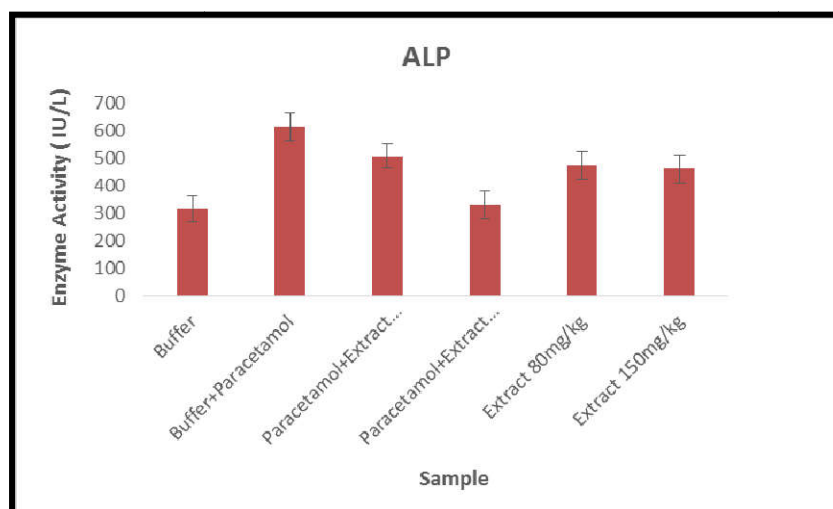


Fig. 5. Significant reduction of GGT activity (IU/L) of paracetamol+extract 150 mg/kg and extract 150 mg/kg in compared with paracetamol. Bars are least significant differences where $p < 0.05$.

Moreover, the rate of GGT decreased significantly in the groups 4, 6 (paracetamol+extract 150 mg/kg, extract 150 mg/kg) in compared with other groups ($P < 0.05$) (Figure 2). These changes were more in the groups treated with the paracetamol+extract 150 mg/kg and extract 150 mg/kg in compared with paracetamol group.

DISCUSSION

According to our investigation, the rate of LDH and GGT was decreased significantly in groups paracetamol+extract 150 mg/kg and extract 150 mg/kg in compared with other groups. In fact, in group paracetamol+extract 150 mg/kg reduced the rate of ALT, AST and ALP in compared with other groups. Whereas, paracetamol group showed the highest ALT, AST, ALP, LDH and GGT activity.

The rise in serum levels of AST, ALT and cholesterol has been attributed to the damaged structural totality of the liver, because they are cytoplasmic in location and released into circulation after cellular damages (27). When rats were treated with poison it induces hepatotoxicity by metabolic activation, therefore, it selectively causes toxicity in liver cells maintaining semi-normal metabolic function. Poison is metabolically activated by the cytochrome P-450 dependent mixed oxidase in the endoplasmic reticulum to form free radical which combined with cellular lipids and proteins in the presence of oxygen to induce lipid peroxidation (28). These result in changes of structures of the endoplasmic reticulum and other membrane, loss of metabolic enzyme activation, reduction of protein synthesis and damage of glucose -6-phosphatase activation, leading to liver injury (29). Treatment with *Francoeuria undulata* aerial parts extract recovered the injured liver to normal at a dose of 150 mg/kg which indicate that *Francoeuria undulata* has antihepatotoxic effect. In addition, the possible antihepatotoxic mechanism of *Francoeuria undulata* have not been reported yet. It is supposed that the effect of *Francoeuria undulata* extract on liver protection is related to glutathione-mediated detoxification as well as free radical repressing activity. In conclusion, from the throughout results of the biochemical examinations.

The involvement of free radicals in the pathogenesis of liver injury has been considered for many years by using acute poisons (30).

The increased activities of liver marker enzymes such as ALT, AST, ALP and LDH in the serum of poisons induced rats demonstrate damage to hepatic cells (31). Damage to the cell totality of the liver by poisons are reflected by an increase in the activity of AST, which is released into circulation after cellular damage. ALP is an ectoenzyme of the hepatocyte plasma membrane. poison-mediated acute toxicity increased permeability of the hepatocyte membrane and cellular exudation (32). This present findings agree with the above reports. The poison-mediated suppression of the increased AST, ALT, ALP and LDH activities suggested the possibility of the extract to give protection against liver injury upon poison induction.

The extracts of leaf and stem bark of *Glycosmis pentaphylla* showed an useful hepatoprotection at a dose of 750 mg/kg in CCl_4 induced rat. A water extract of the root and seed of *Bixa orellana* had been showed hypotensive activity in rat. The same extract showed smooth muscle-relaxant activity in guinea pigs and

lowered gastric secretions in rats which explain its use as a digestive aid and for stomach disturbance (33).

Rezaei *et al.* (2013) reported the significant increases in AST, ALT and ALP with thioacetamide (50 mg/kg) so that, the *A. aucheri* alcoholic extract decreased significantly these parameters at concentrations of 100, 200, and 300 mg/kg. These results were similar to the results of present study at concentration 150 mg/kg (8).

Also, the paracetamol (640 mg/kg) increased the rate of AST and ALT, whereas, the methanolic extract of *A. scoparia* (150 mg/kg) decreased these factors (34).

Kim and Lee (1996) reported *A. selengensis* methanol extract (200 mg/kg) decreased significantly the rate of aminotransferase (35).. While *A. macivera* chloroform extract (50, 100 and 200 mg/kg) increased the rate of AST, ALT and ALP for 60 days. These changes were returned to normal after treatment. They concluded that long-term exposure to this extract is relatively safe but high dose exposure may cause liver damage (36).

CONCLUSION

The result of the present study showed that the high scavenging property of *Francoeuria undulata* aerial parts extract may be due to hydroxyl groups existing in the phenolic compounds' chemical structure that can provide the necessary component as a radical scavenger. Free radicals are often generated as byproducts of biological reactions or poison factors. The involvements of free radicals in the pathogenesis of a large number of diseases are well documented. According to the research, paracetamol may cause oxidative stress in the liver tissue. Moreover, the *Francoeuria undulata* aerial parts extract at concentration 80 mg/kg has toxic effects on liver and this is probably due to the presence of toxic compounds. While, these effects were decreased with increasing of concentration of extract at concentration 150 mg/kg. On the other hand, *Francoeuria undulata* aerial parts extract have the antioxidant and antihepatotoxic effects.

Further work need to be done to isolate and purify the active principle involved in the hepatoprotective activity of this plant. Further study on the plants could be extended for the isolation and structure determination of the hepatoprotective principle or principles.

REFERENCES

- Diallo, D., Hveem, B., Mahmoud, M.A., Betge, G., Paulsen, B.S. & Maiga, A. (1999). An ethnobotanical survey of herbal drugs of *Gourma district*, Mali. *Pharma. Biol.*, 37, 80-91.
- Tomoko, N., Takashi, A., Hiromu, T., Yuka, I., Hiroko, M., Munekazu, I., Totshiuki, T., Tetsuro, I., Fujio, A., Iriya, I., Tsutomu, N. & Kazuhito, W. (2002). Antibacterial activity of extracts prepared from tropical and subtropical plants on methicillin-resistant *Staphylococcus aureus*. *J. Heal. Sci.*, 48: 273-276.
- Sadeghi, H., Jamalpoor, S. & Shirzadi, M.H. (2014). Variability in essential oil of *Teucrium polium* L. of different latitudinal populations. *Indus. Cro. Prod.*, 54: 130-134.
- McKiernan, A.B., O'Reilly-Wapstra, J.M., Price, C., Davies, N.W., Potts, B.M. & Hovenden, M.J. (2012). Stability of plant defensive traits among populations in two *Eucalyptus* species under elevated carbon dioxide. *J. Chem. Eco.*, 38: 204-212.
- Abdel Sattar, E. & Galal, A. M. (1996). Antitumor germacranolides from *Anvillea garcinii*. *J. Nat. Prod.*, 59(4): 403-405.
- Sonboli, A., Babakhani, B. & Mehrabian, A.R. (2006). Antimicrobial activity of six constituents of essential oil from *Salvia zeitschrift*. *J. Biosci.*, 61(3/4): 160-164.
- Thapa, B.R. & Walia, A. (2007). Liver function tests and their interpretation. *Indian. J. Pedia.*, 74: 663-671.
- Rezaei, A., Shekar-Foroush, S.H., Changizi-Ashtiyani, S., Aqababa, H., Zarei, A., Azizi, M. & Yarmahmodi, H. (2013). The effects of *Artemisia aucheri* extract on hepatotoxicity induced by thioacetamide in male rats. *Am. J. Psych.*, 3: 1-9.
- Moujerloo, M. (2010). Variations of lipid peroxidation and superoxide dismutase activity due to haemodialysis in Gorgan. *J. Clin. Diag. Res.*, 4: 2763-2767.
- Ugusman, A., Zakaria, Z., KienHui, C.H., Mohdnordin, N.A.M. & Mahdy, Z.A. 2012. Flavonoids of piper sarmentosum and its cytoprotective effects against oxidative stress. *EXCLI J.*, 11: 705-714.
- Ramarathnam, N.T., Osawa, H., Ochi, S. & Kawakishi, S. (1995). The contribution of plant food antioxidants to human health. *Food. Sci. Tech.*, 6: 75-82.
- Evans, P. & Halliwell, B. (2001). Micronutrients: oxidant/antioxidant status. *Brit. J. Nut.*, 85: 67-74.
- Reische, D., Lillard, W. & Eitenmiller, D.A. (1998). Antioxidants. In *Food Lipids*; Akoh, C.C. Min, D.B. Eds. Marcel Dekker: New York, pp. 423-448.
- Decker, E.A. (1998). Antioxidant machanisms. In *Food Lipids*; Akoh, C.C., Min, D.B., Eds. Marcel Dekker: New York, pp. 397-422.
- Anderberg, A.A., Eldenäs, P., Bayer, R.J. & Englund, M. (2005). Evolutionary relationships in the Asteraceae tribe Inuleae evidenced by DNA sequences of *ndh F*; with notes on the systematic positions of some aberrant genera.

- Organ. Diver. Evol., 5: 135-146.
16. Dendougui, H., Benayache, S., Benayache, F. & Connoly, J.D. (2000). Sesquiterpene lactones from *Pulicaria crispera*. *Fitoter.*, 71: 373-378.
 17. mZareh, M. (2005). Systematic and anatomical studies of Inuleae and Plucheeae in Egypt. *Fedd. Repert.*, 116: 43-53.
 18. Algabr, M.N. (2012). Essential oil composition of *Pulicaria jaubertii* from Yemen. *Inter. J. Med. Arom. Plan.*, 2(4): 688-690.
 19. Amin, G. 2002. Screening of Iranian plants for antifungal activity. *Daru.*, 9(1): 38-48.
 20. Barry, A., Garcia, J. & Thrupp, L.D. (1970). Interpretation of sensitivity test results. *American J. Clin. Patho.*, 53:140-144.
 21. Gering, D.N., Larson, T.A. & Hughes, R.A. (1991). Antimicrobial agents. *Chemotherapy.*, 35: 1284-1286.
 22. Ravandeh, M., Valizadeh, J., Noroozifar, M. & Khorasani-Motlagh, M. (2011). Screening of chemical composition of essential oil, mineral elements and antioxidant activity in *Pulicaria undulata* L. Mey from Iran. *J. Med. Plan. Res.*, 5(10): 2035-2040.
 23. Bohlmann, F., Knoll, K.H. & El-Emary, N.A. (1979). Neuartige sesquiterpene lactone *Pulicaria crispera*. *Phytoche.*, 18: 1231-1233.
 24. Abdel-Mogib, M., Jakupovic, J., Dawidar, A., Metwally, M. & Abou-Elzahab, M. (1990). Sesquiterpene lactones and kaurane glycosides from *Francoeuria crispera*. *Phytochem.*, 29: 2581-2584.
 25. Stavri, M., Mathew, K., Gordon, A., Shnyder, S.D., Falconer, R.A. & Gibbons, S. (2008). Guaianolide sesquiterpenes from *Pulicaria crispera* (Forssk.) Oliv. *Phytochem.*, 69: 1915-1918.
 26. Benzi, I.F, Strain, J.J. (1999). Ferric reducing antioxidant assay. *Methods. Enzymol.*, 292: 15-27.
 27. Sallie, R., Tredger, J.M. & William, R. (1991). Drugs and the Liver. Part I. "Testing liver function". *Biopharm Drug Disp*; 12, pp. 251-259.
 28. Degroot, H. & Noll, T. (1986). The crucial role of low steady state oxygen partial pressures in haloalkan free-radical mediated lipid peroxidation. *Biochem. Pharm.*, 35: 15-19.
 29. Azri, S., Mat, H.P., Reid, L.L., Gandlofi, A.J. & Brendel, K. (1992). Further examination of the selective toxicity of CCl4 rat liver silices. *Toxicol. Apl. Pharm.*, 112(1): 81-86.
 30. Recknagel, R.O., Glende, E.A. Dolak, J.A. & Walker, R.L. (1989). Mechanisms of carbon tetrachloride toxicity. *Pharmacol. Ther.*, 43: 139-54.
 31. Wolf, P.L. (1999). Biochemical diagnosis of liver disease. *Indian. J. Clin. Biochem.*, 14: 59-65.
 32. Paduraru, I., Saramet, A., Danila, G.H., Nichifor, M., Jerca, L. & Iacobovici, A. (1996). Antioxidant action of a new flavonic derivative in acute carbon tetrachloride intoxication. *Eur. J. Drug. Metab. Pharmacokinet.* 21: 1-6.
 33. Dunham, N.W. (1960). A preliminary pharmacologic investigation of the roots of *Bixa orella*. *J. Amer. Pharm. Ass. Sci. Ed.* 49: 218-223.
 34. Gilani, A.H. & Janbaz Kh. (1993). Protective Effect Of *Artemisia Scoparia* Extract Against Acetaminophen-Induced Hepatotoxicity. *Gen. Pharmacol.*, 24: 1455-8.
 35. Kim, K.S. & Lee, M.Y. (1996). Effects of *Artemisia selengensis* methanol extract on ethanol induced hepatotoxicity in rat liver. *J. Korean. Soc. Food. Sci. Nut.*, 25: 581-587.
 36. Atawodi, S.E., Ene, A.C. & Ameh, D.A. (2011). Hepatotoxic effects of chloroform extract of *Artemisia macivera* Linn in rats following intraperitoneal administration of different subchronic doses. *Hum. Exp. Toxicol.*, 30: 25-33.

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